



# BIONETICS

MUTAGENIC EVALUATION OF

COMPOUND 0002992 85

CALCIUM GLUCONATE

(73-5)

Mutagenic Evaluation of Compound FDA 73-5

Calcium Gluconate 4/15/75

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Kensington, Maryland  
20795

LBI PROJECT # 2468

MUTAGENIC EVALUATION OF

COMPOUND 0002992 85

CALCIUM GLUCONATE

(13-5)

SUBMITTED TO

FOOD & DRUG ADMINISTRATION  
-DEPARTMENT OF HEALTH, EDUCATION AND WELFARE  
ROCKVILLE, MARYLAND

SUBMITTED BY

LITTON BIONETICS, INC.  
5516 NICHOLSON LANE  
KENSINGTON, MARYLAND

APRIL 15, 1975



BIONETICS

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### EVALUATION SUMMARY

Compound 000299285, Calcium Gluconate, did not exhibit genetic activity in any of the in vitro microbial assays employed in this evaluation.



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Litton

DATE: 04/15/75

SPONSOR: Food and Drug Administration, Contract Number 223-74-2104

SUBJECT: Evaluation of Test Compound 000299285, Calcium Gluconate

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: August, 1974
2. Description: White, granular powder

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: Saccharomyces cerevisiae, strain D4

Bacteria Strains: Salmonella typhimurium, strains: TA-1535  
TA-1537  
TA-1538

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

<u>Component</u>	<u>Final Concentration/ml</u>
1. TPN (sodium salt)	6 $\mu$ M
2. Isocitric acid	49 $\mu$ M
3. Tris buffer, pH 7.4	28 $\mu$ M
4. $MgCl_2$	1.7 $\mu$ M
5. Tissue homogenate fraction	72 mg

D. Tissue Homogenates and Supernatant

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse-ICR random bred adult males; rat-Sprague-Dawley adult males; and primate-Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1  
POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

<u>Assay</u>	<u>Chemical<sup>a</sup></u>	<u>Solvent</u>	<u>Probable Mutagenic Specificity</u>
Non-activation	Ethylmethane sulfonate	Water or saline	BPS
	2-Nitrofluorene	Dimethylsulfoxide <sup>c</sup>	FS
	Quinacrine mustard	Water or saline	FS
Activation	Dimethylnitrosamine	Water or saline	BPS
	2-Acetylaminofluorene	Dimethylsulfoxide <sup>c</sup>	FS

<sup>a</sup> Concentrations given in the Results Section

<sup>b</sup> BPS = base-pair substitution; FS = frameshift

<sup>c</sup> Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for all chemicals were determined prior to screening.

Each chemical was tested for survival against the specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival curve and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for a chemical with a given strain, then a maximum dose of 5% (w/v) was used against the strain.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

## B. Plate Tests

In the nonactivation procedure, approximately  $10^9$  cells of a log-phase culture of the bacterial indicator strains were spread over the surface of a minimal plate, and a measured amount of the test chemical was placed in the center of the test plate. In activation tests, the test chemical was added to the cells, and an aliquot of the mixture was spread on the surface of the test plate. The reaction mixture (0.1 ml) plus tissue extract was then spotted on the surface of the plate. Positive and solvent controls were included. All plates were incubated at 37°C for four days and then scored. Each compound (test, positive control and solvent control) was done in duplicate. Concentrations of the positive control compounds are listed in the Results Section.

## C. Suspension Tests

### 1. Non activation

Log-phase bacteria and stationary-phase yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of  $1 \times 10^9$  cells/ml and  $5 \times 10^7$  cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic tissue culture plates. Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a  $10^{-1}$  dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

### 2. Activation

Bacteria and yeast cells were grown and prepared as described in the non activation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C in an oxygen atmosphere with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for non activation tests.



D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (sufficient to provide the necessary quantities tissues) were killed by cranial blow, decapitated and bled. Organs were immediately dissected from the animal using aseptic techniques and placed in ice-cold 0.25 M sucrose buffered with Tris at pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies.

E. Data Recording and Reporting

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. Data was then processed and printed from a computer program.



SOLUBILITY PROPERTIES OF THE TEST COMPOUND

1. NAME OR DESCRIPTION OF TEST COMPOUND:

000299 285 Calcium Gluconate

2. TEST SOLVENT AND DESCRIPTION OF SOLUBILITY:

Suspension in 10% Saline  
Not completely soluble at treatment concentrations.

3. OTHER COMMENTS:

White, granular powder



# TOXICITY AND DOSAGE DETERMINATIONS

COMPOUND 000299285

TEST DATE: January 7, 1975

Range of concentrations of the test compound used to determine the 50% survival level

<u>Dose Number</u>	<u>% Concentration</u>
1	10.0
2	1.0
3	0.1
4	0.01
5	0.001

Concentrations of the test chemical required for mutagenicity tests

<u>Dose</u>	<u>% Concentration</u>	
	<u>Bacteria</u>	<u>Yeast</u>
1/4 50% survival	1.25	0.75
1/2 50% survival	2.50	1.50
50% survival	5.00	3.00
Plate Test	2.50	--



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C. Summary of Test Results

Plate Tests

1. Name or code designation of the test compound: 000299285

2. Test date: January 31, 1975

3. Concentration of the test compound: 2.5%

<u>Test</u>	<u>Species</u>	<u>Tissue</u>	<u>TA-1535</u>		<u>TA-1537</u>		<u>TA-1538</u>	
<u>Non-activation</u>			<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>
Solvent Control	--	--	6	1	4	3	6	2
Positive Control <sup>a</sup>	--	--	>10 <sup>4</sup>	>10 <sup>4</sup>	85	74	34	40
Test Compound	--	--	0	5	2	2	4	6
<u>Activation</u>								
Negative Control	--	--	11	12	1	2	12	9
Solvent Control	--	--	4	9	3	1	6	5
Reaction Mixture Control	--	--						
Positive Control <sup>b</sup>	Mouse	Liver	>500	>500	>100	85	>200	>200
Positive Control		Lung	9	5	8	11	15	13
Positive Control		Testes	3	4	7	8	10	9
Positive Control	Rat	Liver	>100	>100	28	24	63	63
Positive Control		Lung	9	4	6	7	12	8
Positive Control		Testes	4	3	8	6	9	12
Positive Control	Monkey	Liver	>100	>100	38	25	31	28
Positive Control		Lung	10	5	6	7	12	6
Positive Control		Testes	4	5	6	6	10	10
Test Compound	Mouse	Liver	4	7	3	1	7	10
Test Compound		Lung	2	5	2	2	6	2
Test Compound		Testes	0	0	3	1	9	6
Test Compound	Rat	Liver	3	6	2	0	5	9
Test Compound		Lung	2	4	2	1	3	2
Test Compound		Testes	0	0	0	1	10	7
Test Compound	Monkey	Liver	3	5	2	0	4	8
Test Compound		Lung	2	4	2	1	3	2
Test Compound		Testes	0	0	0	1	9	6

<sup>a</sup> TA-1535 EMs 10  $\mu$ l/plate  
 TA-1537 QM 20  $\mu$ g/plate  
 TA-1538 NF 100  $\mu$ g/plate

<sup>b</sup> TA-1535 DMNA 50  $\mu$ m/plate  
 TA-1537 AAF 100  $\mu$ g/plate  
 TA-1538 AAF 100  $\mu$ g/plate



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## DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	<p> NAN = Non Activation: Solvent Control  NAP = Non Activation: Positive Control  NA1 = Non Activation: Test Compound Dose 1  NA2, etc. = Reflects the other dose level(s) </p> <p> A+C = Negative Chemical Control  A-C = Activation: Solvent Control  ACP = Activation: Positive Control  ACT = Activation: Test Compound  A+T = Activation: Tissue Control </p> <p> LI = Liver Tissue Activation Fraction  LU = Lung Tissue Activation Fraction  KI = Kidney Tissue Activation Fraction  TE = Testes Tissue Activation Fraction  1,2, etc. = Dose Levels </p>
CONCENTRATION	<p>All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units.</p> <p>Example: 0025-2PCT = 0.25 percent concentration</p>
PGPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = $X 10^6$ ).
MUT 1.	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = $X 10^0$ ). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.



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# DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethyl Methanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey ( <u>Macaca mulatta</u> )
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit



LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/15/75

SPECIES

COMPOUND 000299285

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5
NAN		1.61	12.08	2.92	5.80	2.21	3.14
NAP		243.05	2686.46		469.44	66.33	77.39
NA1		2.23	0.78		2.12	2.05	3.20
NA2		3.17	66.67	2.68	3.04	1.81	3.62

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/15/75

SPECIES ICRFLD COMPOUND 000299285

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5
ACT	A+C	0.93	3.59	4.92	6.30	12.91
ACT	A-C	1.04	3.21	5.77	6.38	13.81
ACT	PLI	128.68	6.71	24.04	9.48	29.12
ACT	PLU	1.00	2.19	7.37	6.67	16.81
ACT	PTE	1.63	2.90	7.02	4.73	17.40
ACT	LII	1.32	3.02	11.39	6.44	24.66
ACT	LI2	1.51	2.70	15.54	7.49	32.06
ACT	LII	1.70	2.41	13.97	5.80	13.06
ACT	LI2	0.84	2.89	13.04	8.92	20.58
ACT	TF1	1.64	3.99	16.99	4.60	14.91
ACT	TF2	1.65	3.46	12.99	7.89	22.66

LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/15/75

SPECIES SPRDAW COMPOUND 000299285

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5
ACT	A+C	2.22	6.77	7.88	5.52	14.73
ACT	A-C	1.45	2.70	6.66	7.34	18.04
ACT	PLI	184.24	44.71	27.00	9.87	21.94
ACT	PLU	2.45	4.96	10.52	6.34	16.64
ACT	PTE	2.83	4.50	8.02	1.63	2.90
ACT	LI1	2.35	15.29	6.82	3.79	16.48
ACT	LI2	0.18	9.89	12.85	6.50	18.50
ACT	LU1	0.75	7.61	2.30	4.31	13.59
ACT	LU2	0.78	4.12	14.85	6.69	21.22
ACT	TE1	1.44	2.67	5.46	4.43	22.78
ACT	TE2	1.98	2.39	13.88	5.06	15.82



LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
REPORT FXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/15/75

SPECIES RHESUS COMPOUND 000299285

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA1538 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5
ACT	A+C	6.27	11.64	8.12		3.21	49.15
ACT	A-C	3.13	0.52	6.60	4.04	5.18	45.95
ACT	PLI	52.59	9.57	24.07		6.74	79.77
ACT	PLU	5.62	5.13	10.43		1.01	68.34
ACT	PTE	6.42	8.91	6.49		3.76	42.38
ACT	LI1	3.14	1.47	20.98	7.34	2.61	25.07
ACT	LI2	3.69	1.38	12.27		5.46	67.47
ACT	LU1	4.26	2.20	10.36		6.19	57.89
ACT	LU2	4.54	1.26	10.80		4.32	51.59
ACT	TF1	3.53	2.47	13.38		3.09	52.26
ACT	TF2	4.29	1.23	9.38		4.14	67.49

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

Compound 000299285, Calcium Gluconate, was evaluated for genetic activity in a series of in vitro microbial assays with and without metabolic activation. The following results were obtained:

A. Salmonella typhimurium

1. Plate tests

At a concentration of 2.5%, 000299285 was not mutagenic for any of the bacteria indicator organisms in either direct or activation plate assays.

2. Nonactivation suspension tests

The results of these tests were negative. The NA2 dose with TA-1537 was high. A repeat test was negative.

3. Activation suspension tests

Some tests using mouse and rat tissues with TA-1538 appeared slightly increased but were not considered positive. One high dose (LII) with primate tissue was repeated. The results were negative. The positive and negative control values for TA-1537 and TA-1538 tests were lower than usual.

B. Saccharomyces cerevisiae

1. Nonactivation suspension tests

The results of these tests were negative.

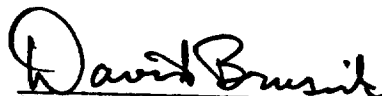
2. Activation suspension tests

The results of these tests were negative. A higher than normal spontaneous background at the TRY locus was observed in these tests.

C. Conclusions

Compound 000299285, Calcium Gluconate, did not exhibit genetic activity in any of the in vitro microbial assays employed in this evaluation.

Submitted by:

  
David Brusick, Ph.D.  
Director of Genetics



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APPENDIX  
Tabulation of Data



**BIONETICS**

REPORT FXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 500902

DETECTOR TA1535

SPECIES

DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUL EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SALINE	1308	0021	1.61	0
	NAP		EMS 0.002 %	1057	2569	243.05	0
000299285	NA1		0025-1 PCT.	0673	0015	2.23	2
000299285	NA2		0125-2 PCT.	0631	0020	3.17	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 502302

DETECTOR TA1537

SPECIES

DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POP1 EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
		NAN	SALINE	0240	0029	12.08	0
		NAP	QM 1.0 UG/ML	0096	2579	2686.46	0
000299285	NA1		0025-1 PCT.	0256	0002	0.78	2
000299285	NA2		0125-2 PCT.	0003	0002	66.67	0 (Repeat)

REPORT FXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 505004

DETECTOR TA1537

SPECIES

DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POP II EP+6	MUT I EP+0	ERF01 EP-8	CONTAM
	MAN		SALINE	2053	0060	2.92	0
000299285	NA2		0125-2 PCT.	2503	0067	2.68	0

REPORT FXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 502301

DETECTOR TA1538

SPECIES

DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POP0 EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		DMSO	0414	0024	5.80	0
	NAP		NF 125 UG-ML	0288	1352	469.44	0
000299285	NA1		0025-1 PCT.	0330	0007	2.12	0
000299285	NA2		0125-2 PCT.	0296	0009	3.04	0

REPORT FXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104			PROJECT 02468						
EXPERIMENT 504801		DETECTOR 000004	SPECIES		DATE - 04/15/75				
COMPOUND	TEST	ORG ID	CONCENTRATION	POPUL EP+4	MUT1 EP+1	MUT2 EP+1	FRF01 EP-5	FRF02 EP-5	CONTAM
	NAN		SALINE	1084	0024	0034	2.21	3.14	0
	NAP		FMS 1.0 %	1004	0666	0777	66.33	77.39	0
000299285	NA1		0015-1 PCT.	1124	0023	0036	2.05	3.20	0
000299285	NA2		0075-3 PCT.	1160	0021	0042	1.81	3.62	4



REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468  
EXPERIMENT 434601 DETECTOR TA1535 SPECIES ICRFLD DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUL EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 50 UM/ML	1286	0012	0.93	0
	A-C		SALINE	1248	0013	1.04	2
	ACP	LI	DMN 50 UM/ML	1210	1557	128.68	0
	ACP	LI	DMN 50 UM/ML	1095	0011	1.00	2
	ACP	TE	DMN 50 UM/ML	1163	0019	1.63	2
000299285	ACT	LI1	0025-1 PCT.	1595	0021	1.32	2
000299285	ACT	LI2	0125-2 PCT.	1395	0021	1.51	2
000299285	ACT	LI1	0025-1 PCT.	1001	0017	1.70	2
000299285	ACT	LI2	0125-2 PCT.	2374	0020	0.84	2
000299285	ACT	TE1	0025-1 PCT.	1404	0023	1.64	2
000299285	ACT	TE2	0125-2 PCT.	1702	0028	1.65	2

REPORT FXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468  
 EXPERIMENT 434701 DETECTOR TA1537 SPECIES ICRFL0 DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POP11 EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	1783	0064	3.59	0
	A-C		DMSO	1590	0051	3.21	0
	ACP	LI	AAF 800 UG/ML	1983	0133	6.71	3
	ACP	LU	AAF 800 UG/ML	1460	0032	2.19	2
	ACP	TE	AAF 800 UG/ML	1619	0047	2.90	2
000299285	ACT	LI1	0025-1 PCT.	1393	0042	3.02	2
000299285	ACT	LI2	0125-2 PCT.	1777	0048	2.70	2
000299285	ACT	LU1	0025-1 PCT.	1701	0041	2.41	2
000299285	ACT	LU2	0125-2 PCT.	1661	0048	2.89	2
000299285	ACT	TF1	0025-1 PCT.	2030	0081	3.99	2
000299285	ACT	TF2	0125-2 PCT.	1848	0064	3.46	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 435001

DETECTOR TA1538

SPECIES ICRFLD

DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUL EP+6	MUT1 EP+0	FRF01 EP-8	CONTAM
	A+C		AAF 800 UG/ML	1363	0067	4.92	0
	A-C		DMSO	1820	0105	5.77	0
	ACP	LI	AAF 800 UG/ML	1252	0301	24.04	3
	ACP	LU	AAF 800 UG/ML	1262	0093	7.37	2
	ACP	TE	AAF 800 UG/ML	1140	0080	7.02	2
000299285	ACT	LI1	0025-1 PCT.	0887	0101	11.39	2
000299285	ACT	LI2	0125-2 PCT.	0920	0143	15.54	0
000299285	ACT	LI1	0025-1 PCT.	0945	0132	13.97	0
000299285	ACT	LI2	0125-2 PCT.	0767	0100	13.04	2
000299285	ACT	TF1	0025-1 PCT.	0865	0147	16.99	0
000299285	ACT	TF2	0125-2 PCT.	1078	0140	12.99	0

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468  
EXPERIMENT 500701 DETECTOR 000004 SPECIES ICRFLN DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0968	0061	0125	6.30	12.91	0
	A-C		SALINE	1050	0067	0145	6.38	13.81	0
	ACP	LI	DMN 90 UM/ML	0728	0069	0212	9.48	29.12	2
	ACP	LU	DMN 90 UM/ML	0809	0054	0136	6.67	16.81	2
	ACP	TE	DMN 90 UM/ML	0931	0044	0162	4.73	17.40	6
000299285	ACT	LI1	0015-1 PCT.	0730	0047	0180	6.44	24.66	6
000299285	ACT	LI2	0075-3 PCT.	0574	0043	0184	7.49	32.06	3
000299285	ACT	LI1	0015-1 PCT.	0827	0048	0108	5.80	13.06	1
000299285	ACT	LI2	0075-3 PCT.	0729	0065	0150	8.92	20.58	0
000299285	ACT	TE1	0015-1 PCT.	0892	0041	0133	4.60	14.91	4
000299285	ACT	TE2	0075-3 PCT.	0887	0070	0201	7.89	22.66	4

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468  
EXPERIMENT 500201 DETECTOR TA1535 SPECIES SPRDAW DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUL EP+6	MUT1 EP+0	FRF01 EP-8	CONTAM
	A+C		DMN 50 UM/ML	0450	0010	2.22	0
	A-C		SALINE	0826	0012	1.45	2
	ACP	LI	DMN 50 UM/ML	0628	1157	184.24	0
	ACP	LU	DMN 50 UM/ML	0653	0016	2.45	0
	ACP	TE	DMN 50 UM/ML	0566	0016	2.83	2
000299285	ACT	LI1	0025-1 PCT.	0767	0018	2.35	2
000299285	ACT	LI2	0125-2 PCT.	0569	0001	0.18	2
000299285	ACT	LI1	0025-1 PCT.	0671	0005	0.75	2
000299285	ACT	LI2	0125-2 PCT.	0644	0005	0.78	2
000299285	ACT	TE1	0025-1 PCT.	0694	0010	1.44	0
000299285	ACT	TE2	0125-2 PCT.	0605	0012	1.98	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 502101

DETECTOR TA1537

SPECIES SPRDAW

DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FRF01 EP-8	CONTAM
	A+C		AAF 800 UG/ML	0251	0017	6.77	0
	A-C		DMSO	0185	0005	2.70	0
	ACP	LI	AAF 800 UG/ML	0170	0076	44.71	2
	ACP	LU	AAF 800 UG/ML	0141	0007	4.96	0
	ACP	TE	AAF 800 UG/ML	0111	0005	4.50	0
000299285	ACT	LI1	0025-1 PCT.	0255	0039	15.29	2
000299285	ACT	LI2	0125-2 PCT.	0556	0055	9.89	2
000299285	ACT	LI1	0025-1 PCT.	0092	0007	7.61	2
000299285	ACT	LI2	0125-2 PCT.	0388	0016	4.12	0
000299285	ACT	TF1	0025-1 PCT.	0300	0008	2.67	2
000299285	ACT	TF2	0125-2 PCT.	0544	0013	2.39	2

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM  
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 500301 DETECTOR TA1538 SPECIES SPRDAW DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUL EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	0964	0076	7.88	0
	A-C		DMSO	1276	0085	6.66	1
	ACP	LI	AAF 800 UG/ML	1052	0284	27.00	0
	ACP	LU	AAF 800 UG/ML	1017	0107	10.52	0
	ACP	TE	AAF 800 UG/ML	1347	0108	8.02	2
000299285	ACT	LI1	0025-1 PCT.	1159	0079	6.82	2
000299285	ACT	LI2	0125-2 PCT.	0887	0114	12.85	2
000299285	ACT	LI1	0025-1 PCT.	1610	0037	2.30	2
000299285	ACT	LI2	0125-2 PCT.	0633	0094	14.85	2
000299285	ACT	TF1	0025-1 PCT.	0806	0044	5.46	2
000299285	ACT	TF2	0125-2 PCT.	0944	0131	13.88	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104				PROJECT 02468					
EXPERIMENT 500801		DETECTOR 000004		SPECIES SPRDAW			DATE - 04/15/75		
COMPOUND	TEST	ORG ID	CONCENTRATION	POPUL EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0706	0039	0104	5.52	14.73	0
	A-C		SALINE	0654	0048	0118	7.34	18.04	0
	ACP	LI	DMN 90 UM/ML	0679	0067	0149	9.87	21.94	0
	ACP	LU	DMN 90 UM/ML	0631	0040	0105	6.34	16.64	0
	ACP	TF	DMN 90 UM/ML	0861	0014	0025	1.63	2.90	0
000299285	ACT	LI1	0015-1 PCT.	0819	0031	0135	3.79	16.48	2
000299285	ACT	LI2	0075-3 PCT.	0600	0039	0111	6.50	18.50	1
000299285	ACT	LI1	0015-1 PCT.	0765	0033	0104	4.31	13.59	2
000299285	ACT	LI2	0075-3 PCT.	0688	0046	0146	6.69	21.22	2
000299285	ACT	TF1	0015-1 PCT.	0632	0028	0144	4.43	22.78	6
000299285	ACT	TF2	0075-3 PCT.	0790	0040	0125	5.06	15.82	6



REPORT FXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468  
 EXPERIMENT 500901 DETECTOR TA1535 SPECIES RHESUS DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POP EP+6	MUT EP+0	FREQ EP-8	CONTAM
	A+C		DMN 50 UM/ML	0734	0046	6.27	0
	A-C		SALINE	1119	0035	3.13	2
	ACP	LI	DMN 50 UM/ML	0945	0497	52.59	3
	ACP	LI	DMN 50 UM/ML	0908	0051	5.62	0
	ACP	TE	DMN 50 UM/ML	0748	0048	6.42	2
000299285	ACT	LI1	0025-1 PCT.	1084	0034	3.14	2
000299285	ACT	LI2	0125-2 PCT.	1137	0042	3.69	2
000299285	ACT	LI1	0025-1 PCT.	0940	0040	4.26	2
000299285	ACT	LI2	0125-2 PCT.	0948	0043	4.54	2
000299285	ACT	TE1	0025-1 PCT.	1076	0038	3.53	2
000299285	ACT	TE2	0125-2 PCT.	1143	0049	4.29	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 502201

DETECTOR TA1537

SPECIES RHESUS

DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUL EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	0146	0017	11.64	0
	A-C		DMSO	0192	0001	0.52	0
	ACP	LI	AAF 800 UG/ML	0188	0018	9.57	0
	ACP	LU	AAF 800 UG/ML	0156	0008	5.13	2
	ACP	TE	AAF 800 UG/ML	0101	0009	8.91	0
000299285	ACT	LI1	0025-1 PCT.	0887	0013	1.47	0
000299285	ACT	LI2	0125-2 PCT.	0435	0006	1.38	2
000299285	ACT	LU1	0025-1 PCT.	0500	0011	2.20	0
000299285	ACT	LU2	0125-2 PCT.	0555	0007	1.26	2
000299285	ACT	TE1	0025-1 PCT.	0364	0009	2.47	0
000299285	ACT	TE2	0125-2 PCT.	0486	0006	1.23	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 501001

DETECTOR TA1538

SPECIES RHESUS

DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUL FP+6	MUT1 FP+0	FRF01 EP-8	CONTAM
	A+C		AAF 800 UG/ML	0936	0076	8.12	0
	A-C		DMSO	1076	0071	6.60	2
	ACP	LI	AAF 800 UG/ML	0810	0195	24.07	3
	ACP	LU	AAF 800 UG/ML	1064	0111	10.43	0
	ACP	TF	AAF 800 UG/ML	1263	0082	6.49	2
000299285	ACT	LI1	0025-1 PCT.	0572	0120	20.98	3 (Repeated)
000299285	ACT	LI2	0125-2 PCT.	0880	0108	12.27	2
000299285	ACT	LU1	0025-1 PCT.	0676	0070	10.36	2
000299285	ACT	LU2	0125-2 PCT.	0954	0103	10.80	2
000299285	ACT	TF1	0025-1 PCT.	0785	0105	13.38	0
000299285	ACT	TF2	0125-2 PCT.	1045	0098	9.38	2

REPORT FXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468  
EXPERIMENT 505003 DETECTOR TA1538 SPECIES RHESUS DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPUL EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A-C		DMSO	0570	0023	4.04	0
000299285	ACT	LI1	0025-1 PCT.	0518	0038	7.34	0

REPORT EXR33 LITTON BIOGENETICS MUTAGENIC ACTIVITY SYSTEM  
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468  
EXPERIMENT 502901 DETECTOR 000004 SPECIES RHESUS DATE - 04/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POP11 EP+4	MUT1 EP+1	MUT2 EP+1	ERF01 EP-5	ERF02 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0529	0017	0260	3.21	49.15	4
	A-C		SALINE	0618	0032	0284	5.18	45.95	0
	ACP	LI	DMN 90 UM/ML	0341	0023	0272	6.74	79.77	0
	ACP	LU	DMN 90 UM/ML	0398	0004	0272	1.01	68.34	2
	ACP	TE	DMN 90 UM/ML	0505	0019	0214	3.76	42.38	0
000299285	ACT	LI1	0015-1 PCT.	1073	0028	0269	2.61	75.07	0
000299285	ACT	LI2	0075-3 PCT.	0458	0025	0309	5.46	67.47	0
000299285	ACT	LU1	0015-1 PCT.	0323	0020	0187	6.19	57.89	0
000299285	ACT	LU2	0075-3 PCT.	0440	0019	0227	4.32	51.59	0
000299285	ACT	TE1	0015-1 PCT.	0421	0013	0220	3.09	52.26	0
000299285	ACT	TE2	0075-3 PCT.	0483	0020	0326	4.14	67.49	2